Appln. No. 10/593,265 Amd. dated June 14, 2011

Reply to Office Action of February 15, 2011

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) Genetically A genetically modified plants, characterized in that they include comprising one or more than one copy of at least a two different sequences encoding two different a eukaryotic P_{1B} -type ATPase of the $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$ subclass and that they overexpresses said two different P_{1B} -type ATPase.

2. (**Currently Amended**) The Ggenetically modified plants according to claim 1, characterized in that wherein said P_{1B} -type ATPase is selected from the group consisting of heavy metal ATPase HMA1, HMA2, HMA3 and HMA4 of *Arabidopsis thaliana*.

3. (Cancelled)

4. (Currently Amended) The Genetically modified plants according to claim 1, characterized in that they wherein the genetically modified plant further includes one or more than one copy of a sequence encoding a P_{1B}-type ATPase of the Zn²⁺/Co²⁺/Cd²⁺/Pb²⁺-subclass and at least another sequence selected among sequences encoding (1) an enzyme involved in metal chelation (phytochelatin synthase, glutathion synthese or gamma-glutamylcystein synthase) or and (2) another metal transporter such as YCF1 or other ABC transporters.

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5. (Currently Amended) Recombinant A recombinant vector able to transform plants, characterized in that wherein said vector includes one or more than one copy of at least two different a sequences encoding two different eukaryotic a P_{1B} -type ATPase of the $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$ subclass.

6. (**Currently Amended**) The Rrecombinant vector according to claim 5, characterized in that wherein said coding sequences are operably linked to and under the regulatory control of a plant-expressible transcription and translation regulatory sequence, such as a plant specific promoter.

7. (**Currently Amended**) <u>A G</u>genetically modified plants, characterized in that they are that has been transformed with a recombinant vector according to claim 5.

8. (**Currently Amended**) Plant cells characterized in that they are transformed with a recombinant vector according to claim 5.

9. (**Currently Amended**) <u>A Mm</u>ethod of producing genetically modified plants according to claim 1 which overexpress<u>es</u> at least <u>two different eukaryotic</u> a P_{1B} -type ATPase of the $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$ subclass, said method comprising:

- preparing a recombinant vector according to claims 5-or 6, comprising one or more than one copy of at least a-two different sequences encoding two different eukaryotic a P_{1B} -type ATPase of the $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$ subclass, operably linked to

and under the regulatory control of a plante-expressible transcription and translation regulatory sequence and

- introducing said recominant vector into a plant cell or plant tissue to produce a genetically modified plant cell or a genetically modified plant tissue.
- 10. (**Currently Amended**) <u>A Mm</u>ethod of phytoremediation of heavy metals from soil, <u>comprising</u> characterized in that it includes:
- a step of planting genetically modified plants according to claim 1, in an area containing soil contaminated with at least one heavy metal and
- collecting and removing plant tissues from said genetically modified plants at appropriate time intervals.
- 11. (**Currently Amended**) Method The method of phytoremediation according to claim 10, characterized in that it involves the extraction of one of the following heavy metals: wherein Zn, Co, Cd or Pb, is extracted from soil.
- 12. (**Currently Amended**) The Mmethod of phytoremediation according to claim 10, characterized in that wherein the entire plant might be is removed after it is allowed to grow on metal-containing soil incorporating those metals into its tissues.
- 13. (**Currently Amended**) Method The method of phytoremediation according to claim 10, characterized in that wherein at appropriate time intervals, the metal containing tissues and more preferably leaves and possibly branches are removed from the plant, allowing the remaining plant tissues to survive.

14. (**Currently Amended**) Method The method of phytoremediation according to claim 13, characterized in that wherein the collected plant tissues are removed from the growing area and properly disposed, so that the metal containing tissues are not allowed to reassimilate in the soil.

15. (Currently Amended) $\underline{\text{Method-}}\underline{\text{The method}}$ of phytoremediation according to claim 13, $\underline{\text{characterized in that}}\underline{\text{wherein}}$ said heavy metals may be extracted, in the M^{n+} state, from said plant tissues.

16. (**Currently Amended**) Method The method of phytoremediation according to claim 13, characterized in that wherein said heavy metals are extracted from ashes obtained after having burnt the collected metal containing tissues, said metal being in the M⁰ state.

17. (Currently Amended) <u>A G</u>genetically modified plants, as defined in claim 1, <u>characterized in that wherein</u> said plants <u>are is</u> selected in the group consisting of *Brassica juncea*, *Poplar*, *Nicotiana tabacum*.

18. (**Previously Presented**) A method for phytoextracting Zn, Co, Cd or Pb, from a contaminated environment comprising planting a genetically modified plant according to claim 1.

19. (**Previously Presented**) A method for phytoextraction of Co, Cd or Pb, from a contaminated environment comprising planting a genetically modified plant according to claim 7 in the contaminated environment.

- 20. (**Currently Amended**) <u>A Mm</u>ethod of producing genetically modified plants according to claim 1, which plants overexpress at least <u>two different eukaryotic a</u> P_{1B} -type ATPase of the $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$ subclass, said method comprising:
- preparing at least one recombinant vector(s) according to claims 105 or 6, comprising one or more than one copy of at least $\underline{\text{two different a}}$ sequences encoding $\underline{\text{two different eukaryotic a}}$ P_{1B} -type ATPase of the $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$ subclass, operably linked to and under the regulatory control of a plante-expressible transcription and translation regulatory sequence and
- introducing said at least one recominant vector(s) into a plant cell or plant tissue to produce a genetically modified plant cell or a genetically modified plant tissue.
- 21. (**Currently Amended**) A method of phytoremediation of heavy metals from soil, comprising:
- planting <u>a</u> genetically modified plants according to claim 1, in an area containing soil contaminated with at least one heavy metal and
- collecting and removing plant tissues from said genetically modified plants at appropriate time intervals.
- 22. (**Currently Amended**) The method of phytoremediation according to claim 21, wherein the method involves the extraction of at least one of the following heavy metals: Zn, Co, Cd or and Pb, from soil.

- 23. (**Previously Presented**) The method of phytoremediation according to claim 21 wherein the entire plant is removed after it has been allowed to grow on metal-containing soil.
- 24. (**Previously Presented**) The method of phytoremediation according to claim 21, wherein at appropriate time intervals, metal containing tissues are removed from the plant, said plant being left alive.
- 25. (**Currently Amended**) The method of claim 24, wherein leaves and optionally branches of the plant are removed.
- 26. (**Previously Presented**) The method of phytoremediation according to claim 24, wherein the collected plant tissues are removed from the growing area and properly disposed, so that the metal containing tissues are not allowed to reassimilate in the soil.
- 27. (**Previously Presented**) The method of phytoremediation according to claim 24, wherein said heavy metals are extracted, in the M^{n+} state, from said plant tissues.
- 28. (**Previously Presented**) The method of phytoremediation according to claim 24, wherein said heavy metals are extracted from ashes obtained after having burnt the collected metal containing tissues, said metal being in the M⁰ state.
- 29. (New) A genetically modified plant according to claim 1, characterized in that said P_{1B} -type ATPase of the $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$ subclass is from a higher plant.

- 30. (New) A genetically modified plant according to claim 4, characterized in that said enzyme involved in metal chelation is selected from the group consisting of phytochelatin synthase, glutathion synthetase and gamma-glutamylcystein synthase, and/or said metal transporter is selected from the group consisting of YCF1 and an ABC transporters.
- 31. (**New**) A recombinant vector according to claim 6, characterized in that said plant-translation regulatory sequence is a plant specific promoter.
- 32. (**New**) The method according to claim 9, characterized in that said enzyme involved in metal chelation is selected from the group consisting of phytochelatin synthase, glutathion synthetase and gamma-glutamylcystein synthase, and/or said metal transporter is selected from the group consisting of YCF1 and ABC transporters.
- 33. (**New**) The method of claim 13, characterized in that said tissues are leaves and/or branches.
- 34. (**New**) The method according to claim 20, characterized in that said enzyme involved in metal chelation is selected from the group consisting of phytochelatin synthase, glutathion synthetase and gamma-glutamylcystein synthase, and/or said metal transporter is selected from the group consisting of YCF1 and ABC transporters.